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14. ABSTRACT
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Principal Investigator	Edward J. Kramer (with Christopher K. Ober)
Organization(s)	University of California at Santa Barbara & Cornell University

Abstract

This program seeks to understand the spatial and temporal chemistry required for new antifouling/fouling release polymer coatings from chemically patterned surfaces with different time responses to their environment. Our work focuses on preparing and testing new polymers with amphiphilic structures. Here we summarize several novel amphiphilic polymers prepared in the past year as well as a novel polymer brush system which allows us to produce chemically patterned surfaces for biofouling assays. This new brush patterning system will allow us to test the effect of chemical pattern length scales on the settlement of marine organisms. Those polymers have been synthesized and fully characterized. Glass slides or silicon wafers are coated with polymers through optimized multilayer processes established in our labs and then the surface properties are analyzed. Surface analytical methods include imaging X-ray photoelectron spectroscopy (XPS), near-edge X-ray absorption fine structure (NEXAFS), atomic force microscopy (AFM) and neutron reflection (NR) as well as water and/or bubble contact angle studies. Such measurements provide essential feedback to allow us to discern the connections between the molecular connectivity and microstructure of our surfaces and their fouling resistance/release. *In situ* neutron reflectivity measurements have given us new insight into the structural rearrangements of hydrophobic polymer brushes when immersed in water. In addition, studies of those functional polymer coated surfaces with ONR investigators such as M. Callow, D. Wendt and M. Hadfield provided direct evaluation of the antifouling performance of these surfaces. Surfaces coated with our specialized polymers have been evaluated using settlement and release assays involving *Navicula* and *Ulva Linza*. Several polymer coatings showed comparable, in some cases better, antifouling and fouling release properties than traditional PDMS-based coatings. Those results have also shown that modifying existing materials with amphiphilic functionalities may provide a promising approach to produce novel environmentally benign antifouling materials for surface coating, and the results can also provide insight into the rational design of the next generation of antifouling and fouling release materials.

Technical Section

Technical Objectives

Marine biofouling on naval vessels is a persistent and highly undesirable problem. The increase in roughness imparted by the accumulation of biofouling increase drag and leads to substantial energy and security penalties, and requires frequent maintenance to remove. Often antifouling/fouling release coatings are needed to protect those engineered structures. Historically, toxic antifoulants have been used as a method of controlling fouling. However, toxic biocides such as tin, copper, lead, arsenic, mercury and their organic derivatives (such as organotins) have been banned in many countries due to the environmental risks they posed. The leached toxins accumulate in marine environments, especially near ports and harbors, and are dangerous for non-target marine organisms and humans by partitioning of metabolic functions. In recent years, with increased legislation on toxicity requirements, the research community has been actively exploring and developing new, robust, and environmentally benign alternative coating materials that inhibit attachment and reduce adhesion strength to prevent biofouling. Particularly, polymer coatings incorporating amphiphilic structures have shown some remarkable characteristics and received considerable attention. It is suggested that since they contain both hydrophobic and hydrophilic components, they can form "ambiguous surfaces". Therefore, they may offer a solution to produce a universal antifouling/fouling release coating capable of resisting settlement as well as releasing a wide variety of fouling organisms. Amphiphilic copolymers containing perfluorocarbon and PEG side chains have been evaluated using settlement and release assays of *Navicula* diatoms and *Ulva Linza* spores. The results showed comparable or improved antifouling and release properties over traditional PDMS-based coatings. However, up until now there is little understanding of why these systems work, thus hindering attempts to rationally design coatings with even better antifouling/fouling release properties. For extended performance life, such coating systems must have controlled and consistent surface energy and composition (both polar and/or non-polar), have both elastomeric properties and good adhesion to a selected substrate. To date, several commercial antifouling/fouling release coating systems are on the market, all based on silicone technology, yet none meet all of the desired performance characteristics. Many lack the toughness required to stand up to the rigorous physical demands of the marine environment, do not sufficiently and consistently self-clean, or due to polymer re-structuring or degradation, lose many of the desirable surface properties with time and exposure to the complex and dynamic marine environment.

The goal of our research is to develop novel non-toxic, easily processable, environmentally benign antifouling and/or fouling-release marine coatings with improved performance compared to currently utilized copper ablative and PDMS based coatings, proving effective against as wide a spectrum of fouling organisms as possible. Our effort focuses in particular on improved amphiphilic polymer coatings based on poly(styrene)$_{8k}$-*block*-poly(ethylene-ran-butylene)$_{25k}$-*block*-poly(isoprene)$_{10k}$ (PS-*b*-P(E/B)-*b*-PI) triblock copolymer, P(DMS-*stat*-MVS) block copolymers, polystyrene-*block*-poly(ethylene oxide–*stat*-allyl glycidyl ether) [PS-*b*-P(EO-*stat*-AGE)] block copolymers, and P(EO-*stat*-AGE) brushes. As part of this effort, we also explore new functional segments based on oligopeptides, and use those polymers to prepare surfaces with chemical structures at various length scales. This will allow us to develop an improved understanding of the spatial length scales and temporal environmental response that lead to such high performance of "ambiguous" coatings. The results can provide insights to create better antifouling and fouling release materials in the future through rational materials design targeting known effective structures.

Technical Approach

Our approach is to synthesize and examine amphiphilic surface active block copolymers for antifouling/fouling release coatings through careful material and surface analysis, in addition to marine organism testing. These amphiphilic structures, prepared by the Ober and Kramer groups in collaboration with Rachel Segalman and Craig Hawker, include those derived from combinations of hydrophilic PEG materials and hydrophobic units (fluorinated, hydrocarbon, peptide, peptoid and silicone groups). To apply the synthesized block copolymer on the substrates, our approach is to use bilayer coatings based on commercially sourced polystyrene-*block*-poly(ethylene-*ran*-butylene)-*block*-polystyrene (SEBS) thermoplastic elastomers. A thermoplastic elastomer (TPE) forms the bulk of the coating, providing necessary mechanical properties with respect to both durability and bulk modulus. Meanwhile, a relatively thin layer of the specially designed surface-active block copolymer (SABC) is spray-coated on top of the elastomeric layer and modifies the surface chemical and physical properties. In this work, we also aim to prepare polymer brushes from silane initiators on the surface; these brushes and the brushes will be patterned using UV lithographically activated "thiol-ene" chemistry to attach a pattern of desired chemical structures. This patterning allows us to apply highly controlled chemical patterns directly to a surface. Surface analysis is important in providing molecular level information about material behavior and their biological activities. Studies by the Kramer and Ober groups include time and spatially resolved X-ray photoelectron spectroscopy (XPS), near edge X-ray absorption fine structure (NEXAFS), atomic force microscopy (AFM) and neutron reflection (NR) measurements as well as contact angle studies to analyze those surfaces. All the polymer coatings are then tested for settlement and adhesion strength of marine algae and other organisms available through collaboration with several ONR PIs, in particular by the Callow group, the Wendt group and the Clare group.

Progress Statement Summary

In the past year, our work has been focused on finding new amphiphilic polymeric systems for marine antifouling applications using perfluorocarbons, saturated hydrocarbons and silicone based materials. Three major polymer systems have been developed and examined here. The first system involves the grafting of hydrocarbon (hydrophobic) and polyethylene glycol (PEG) (hydrophilic) amphiphilic surfactants on PS-*b*-P(E/B)$_k$-*b*-PI (or K3) triblock copolymers. The second amphiphilic system contains PDMS polymer backbone (PS-*b*-P(DMS-*stat*-MVS) as hydrophobic segments and PEG or oligopeptide side chains as hydrophilic segments. The third system focuses in particular on improved amphiphilic coatings derived from polystyrene-*b*-poly(ethylene oxide-*stat*-allyl glycidyl ether) [PS-*b*-P(EO-*stat*-AGE)] block copolymers. In the latter two polymer systems, short vinyl groups were incorporated to introduce a broad range of selected thiols using a thiol-ene "click" reaction triggered by UV light. In addition, we explored some very interesting pendent thiol ligands such as oligopeptides. The chemical structures of all the synthesized polymers were confirmed using NMR, IR, GPC and elemental analysis. The polymer coated surfaces were also characterized using advanced characterization techniques including near edge X-ray absorption fine structure (NEXAFS) spectroscopy, X-ray photoelectron spectroscopy (XPS), neutron reflection (NR), atomic force microscopy (AFM) and air bubble/water contact angle studies. Some of the final polymers were tested against both *Ulva* and *Navicula,* the results demonstrated that, at certain chemical compositions, better antifouling/fouling release behavior than standard PDMSe polymers can be established for both organisms.

Progress

In this report, we describe synthesis, characterization and the antifouling assays of fluorine-free, non-ionic hydrocarbon and PEG amphiphilic side chains modified PS-*b*-P(E/B)-*b*-PI polymers. In addition, we introduce new polymer systems based on PS-*b*-P(DMS-*stat*-MVS) block copolymers and polystyrene-*b*-poly(ethylene oxide–*stat*-allyl glycidyl ether) [PS-*b*-P(EO-*stat*-AGE)] brushes. We also describe synthesis and characterization of short chain oligopeptides and how to use them to functionalize those polymers. Moreover, currently we are investigating the effect of chemical patterning by fabricating stripes and other patterns of fluorinated and PEGylated on PS-*b*-PAGE and PS-*b*-P(EO-*stat*-AGE) brush surfaces. The settlement and attachment strengths of the marine algae *Ulva* and *Navicula* of some of these polymers and brushes are currently under investigation.

1) Patterned Surfaces. Surfaces patterned with hydrophobic and hydrophilic stripe domains were successfully produced with dimensions from 50 to 2 microns, using UV photolithography.

These patterns were achieved by functionalizing end tethered PAGE polymer brushes with either the hydrophobic 1H, 1H, 2H, 2H perfluorododecanethiol (PFDT) or the hydrophilic thiol terminated polyethylene glycol side chains using UV activated thiol-ene click reaction. These surfaces were tested against Ulva zoo spore settlement and the results indicate that the Ulva settle in a stripe pattern determined by the underlying hydrophobic/hydrophilic chemical pattern. The reorganization of the PAGE brushes functionalized with PFDT when immersed in water was also investigated using neutron reflectivity. It was found that the PFDT functionalized brushes drastically rearrange themselves when immersed in water and that part of this rearrangement appears to not recover when the brush is exposed to air or even when it is annealed at 90 °C. This work has revealed important information about how hydrophobic polymer brushes rearrange when submersed in water. Future work will investigate how amphiphilic polymer brushes for instance P(EO-b-fAGE) brushes rearrange.

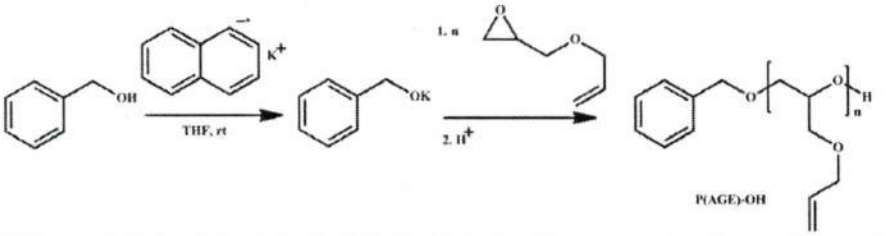
2) Settlement and release of marine algae from PDMS-block-PS surfaces with amphiphilic surface-active additives. Amphiphilic block copolymers were prepared containing a polydimethylsiloxane (PDMS) block and a statistical copolymer of a poly(ethylene glycol) functionalized methacrylate (PEGMA) and a fluoroalkyl acrylate (AF6) prepared by ATRP. The statistical block of PEGMA and AF6 was varied in polymer chain length as well as relative composition of hydrophilic (PEGMA) and hydrophilic (AF6) components. This allowed for a systematic study of the effects of composition of the block copolymer on the performance of the antifouling coatings. The surfaces were prepared by using a crosslinked PDMS matrix loaded with the amphiphilic block copolymers at two different mass percentages. These crosslinked coatings provide a tough and elastic surface for performing *Ulva* settlement and attachment testing. Several of the surfaces exhibited superb release of *Ulva* sporelings at very low removal shear stress relative to a PDMS standard, and even exhibited “spontaneous removal” of sporelings during the incubation period. Overall the best performing coatings contained a low molecular weight amphiphilic block copolymer additive and a ratio of PEGMA to AF6 of 50:50.

3) Settlement and release of marine algae from PS-*b*-P(DMS-*stat*-MVS) diblock copolymer with grafted unnatural oligopeptide surfactants. Unnatural oligopeptides surfactants, modified with hydrocarbon and PEG chains, were grafted to polystyrene-block-poly(dimethylsiloxane-stat-methylvinylsiloxane) [PS-*b*-P(DMS-*stat*-MVS)] diblock copolymers. The synthesized polymers (block and alternating) were confirmed using ^{1}HNMR. These polymers were spray coated onto glass slides and tested against *Ulva*. It was determined that the *Ulva* settlement and growth was lower on the modified PS-*b*-P(DMS-*stat*-MVS) diblock copolymer than the unmodified control or the PDMSe standard. The percent removal of the *Ulva* from the surface for the modified diblock copolymers was much higher than the control, but it was lower than the PDMSe standard. The alternating and the block oligopeptide patterns had similar results to each other in all categories.

4) Settlement and release of marine algae and diatoms from PS-b-P(E/B)-b-PI triblock copolymer with grafted hydrocarbon/PEG amphiphilic surfactants. Two fluorine-free hydrocarbon/PEG surfactants were grafted to poly(styrene)-*block*-poly(ethylene-*ran*-butylene)-*block*-poly(isoprene) (PS-*b*-P(E/B)-*b*-PI) triblock copolymers. The synthesized polymers (K3-HC-PEG350 and K3-HC-PEG550) were confirmed with various techniques and both polymers were spray coated onto SEBS treated glass slides. Surface analysis with bubble contact angles showed these surfaces can undergo surface reconstruction when exposed to water and the process reaches equilibrium after three days. Antifouling/fouling release performance of these polymeric coatings against *Ulva* and *Navicula* were also tested. Compared to standard PDMSe coated surfaces, polymer coatings with longer PEG chains (K3-HC-PEG550) showed less cell attachment for both species, with release of *Ulva* up to 100 % and less remaining *Navicula*, indicating those polymers as potential antifouling/fouling release coatings in marine environment.

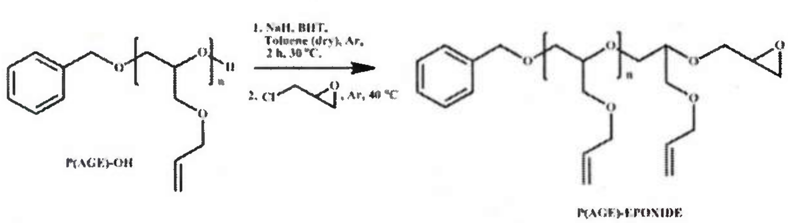
5) General Procedure for Preparation of Epoxide terminated PAGE

A representative polymerization procedure was as follows: the polymerization was carried out on a Schlenk line in custom thick-walled glass reactors fitted with ACE-threads under an argon atmosphere. The reactors were dried under vacuum then refilled with argon five times. Under an argon atmosphere, benzyl alcohol initiator was added by gas-tight syringe through a 6-mm puresep septum. Potassium alkoxide initiators were formed by titration of benzyl alcohol with potassium naphthalenide under argon until a green color persisted in solution indicating the deprotonation of all alcohols. Solution polymerizations were carried out in dry THF at room temperature for 45 h and terminated with methanol. The reaction conditions has been shown to result in low polydispersity (PDI=1.08), controlled molecular weight materials (8 kg/mol). PAGE homopolymer was eluted from silica by hexane. This could be used for separation of the presence contaminants due to initiating step. Then, PAGE homopolymer were eluted with ethyl acetate. The viscous colorless oil was dried in vacuo before characterization.



Scheme 1. Polymerization of allyl glycidyl ether from potassium benzoxide.

Synthesis of epoxide terminated PEG, PEG-EP was prepared according to literature [1] procedures with a few modifications. PAGE was dried under vacuum at room temperature for 24 h and then dissolved with 20 mL of fresh distilled toluene. A catalytic amount of BHT as a radical inhibitor was added. NaH (1.1 equiv) was introduced under the protection of argon flow at room temperature. After H_2 evolution for about 10 min, the mixture was sealed under argon and stirred at 30 °C for 3 h. Epichlorohydrin (5 equiv) was then added. The mixture was stirred at 40 °C for 5 days and then precipitated into an excess of cold diethyl ether. The crude product was dissolved with CH_2Cl_2 (100 mL) and washed with distilled water (50 mL) three times. The organic phase was dried over anhydrous Mg_2SO_4. After filtration, the solution was concentrated by rotary evaporation and precipitated into cold diethyl ether. The product was wash with diethyl ether three times and dried under vacuum. Yield: 86.3%.

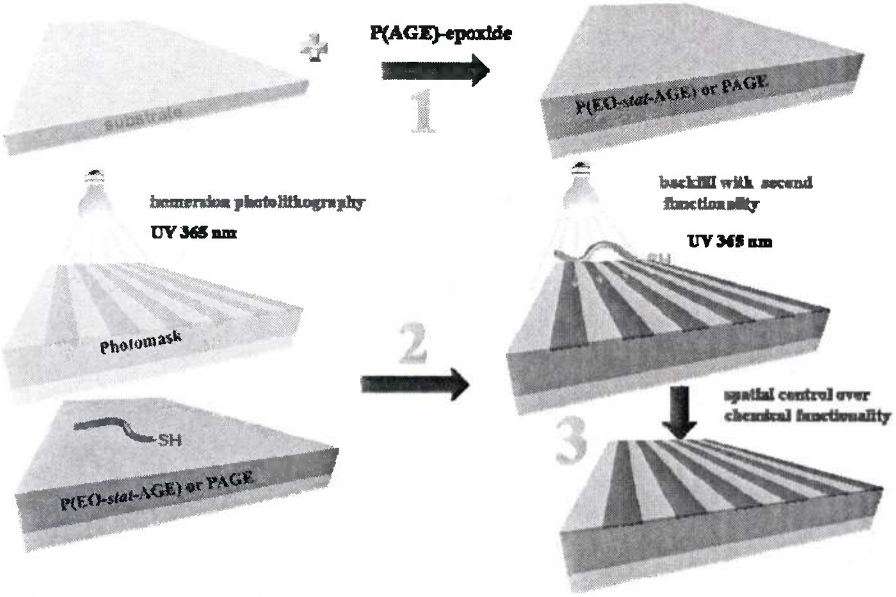


Scheme 2. Synthesis of epoxide end-functionalized P(AGE).

Experimental Approach

1) Patterned Surfaces

Patterned surfaces were made using the procedure summarized in scheme 3 below. Silicon wafers were cleaned using piranha solution for 30 minutes in order to produce silanol groups at the native silicon dioxide surface layer. Next a 3-aminopropyltrimethoxysilane (APTMS) layer, approximately 20 nm thick, was spun coat at 3000 rpm upon the silanized silicon wafers, from a solution of ethanol and water with a pH adjusted to 4.5 with acetic acid. The APTMS coated silicon wafers were annealed for ~12 hrs at 90 °C to crosslink the layer. Next epoxy terminated PAGE was reacted with the amine groups of the APTMS layers, using the "polymer melt grafting to" approach in order produce a surface coated with end tethered PAGE polymers. This was achieved by simply squeezing a few drops of the low Tg PAGE polymer (liquid at room temp), between two APTMS coated silicon wafers. These wafers were then placed in the oven at 80 °C for 12 hrs. Note, the free radical quencher butylated hydroxytoluene (BHT) was dissolved in the epoxy terminated PAGE before annealing, in order to prevent heat induced crosslinking of the PAGE polymer. An elevated temperature of 80 °C was primarily chosen to increase the rate of the epoxy-amine reaction, as well as to increase the mobility of the PAGE polymers. The PAGE brush coated surfaces were then patterned using (365 nm) UV click chemistry with 1H, 1H, 2H, 2H perfluorododecanethiol (PFDT) (hydrophobic) and 2k M_w PEG-SH (hydrophilic). Striped patterns were achieved by using chrome on quartz photolithographic masks with stripes of width 50, 20, 10, 5 and 2 microns. It was found that it was necessary to add 0.5% by wt BHT to the UV click solutions in order to prevent lateral diffusion of thiol radicals into areas of the PAGE coated wafers which were not irradiated with UV light.

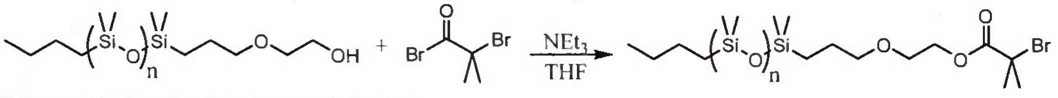


Scheme 3. General method for production of patterned surfaces.

2) Synthesis of PDMS based amphiphilic copolymer surfaces

All copolymers were synthesized using 10,000 g/mol hydroxyl-terminated PDMS. 5g (0.5mmol) of the starting material was degassed under vacuum by aspiration for 30 minutes before use. The polymer was then dissolved in ~10mL of distilled tetrahydrofuran (THF). To this solution 278.8μL (2mmol) of distilled triethylamine (NEt_3) was added and allowed to stir for 15 minutes at room temperature. To this solution 123.6μL (1mmol) of α-bromo butyryl bromide dissolved in

5mL of distilled THF slowly was dripped in under nitrogen. The reaction was stirred under nitrogen for 72 hours. At this time the THF was removed by rotary evaporation. The impure polymer was then dissolved in dichloromethane (DCM) and the solution was washed 5 times each with 1M hydrochloric acid, concentrated potassium bicarbonate and deionized water. The solution was then dried using sodium sulfate, and the DCM was removed by rotary evaporation.



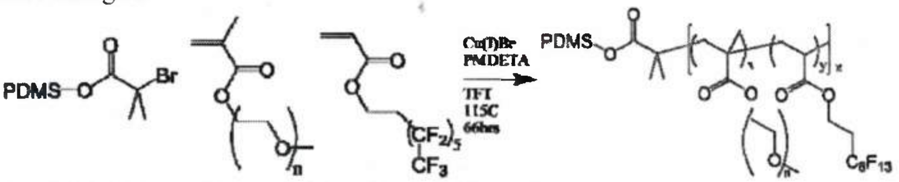
Scheme 4: Synthesis of macroinitiator

All polymerizations performed were atom transfer radical polymerization (ATRP) reactions. All polymerizations were carried out under the same conditions, only modifying the amount of the monomers (3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl acrylate (AF6) and polyethylene glycol methyl ether methacrylate (PEGMA) of molecular weight Mn ~ 300g/mol) added to each reaction to achieve different copolymers. 1.2g (0.12mmol) of the macroinitiator was added to a Schlenk tube along with 5.5mL or trifluorotoluene (TFT) and 25.06µL (0.12mmol) of pentamethyldiethylenetriamine (PMDETA). Monomer was added at nine different ratios broken into three sets. The first set was targeted to produce copolymers with a monomer to initiator ratio of 30:1, the second set at 60:1 and the third set at 150:1. The ratio between the monomers (AF6:PEGMA) for each set was 70:30, 50:50, and 30:70. The volumes are listed in the table below:

	30:1 Monomer Loading			60:1 Monomer Loading			1S0:1 Monomer Loading		
AF6: PEGMA	30:70	S0:S0	70:30	30:70	S0:S0	70:30	30:70	S0:S0	70:30
AF6	290µL	484µL	867µL	S80uL	968µL	13S6µL	14S0µL	2421µL	3389µL
	1.08mmol	1.80mmol	2.S2mmol	2.16mmol	3.60mmol	S.04mmol	S.40mmol	9.00mmol	12.60mmol
PEGMA	720µL	S14µL	309µL	1440µL	1029µL	618µL	3600µL	2S72µL	1S4SµL
	2.S2mmol	1.80mmol	1.08mmol	S.04mmol	3.60mmol	2.16mmol	12.60mmol	9.00mmol	S.40mmol

Table 1: Monomer Loading Volumes for Polymerizations

Once all of these components were added, the mixture was Freeze-Pump-Thawed three times to remove oxygen and replace with nitrogen. After these three cycles, 17.21mg (0.12mmol) of Cu(I)Br was added to the reaction. The solution was Freeze-Pump-Thawed a further four times, and became blue in color. Then the reactions were heated to 115°C and allowed to react for 66 hours under nitrogen.

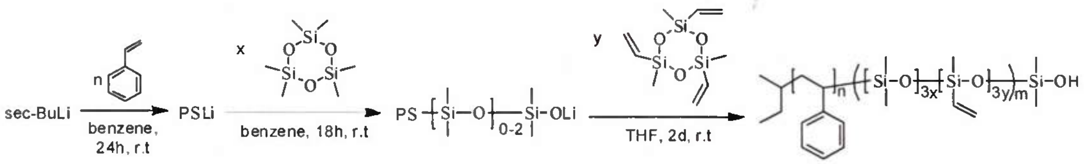


Scheme 5: ATRP Polymerization of Amphiphilic Block Copolymer

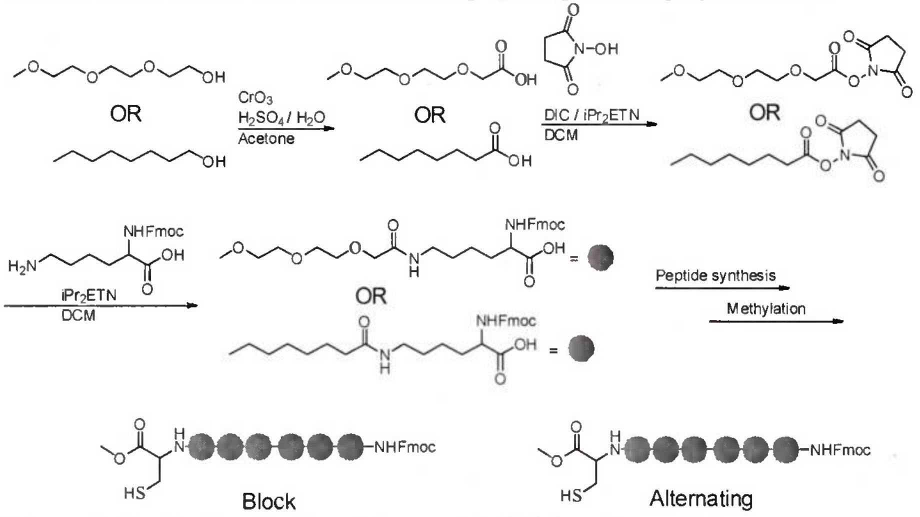
After the completion of the reaction, the dichloromethane was added to the reaction solution The solution was then washed 3 times with potassium bicarbonate solution, then 4 times with deionized water to remove copper and other impurities. The solution was dried with sodium sulfate, and the DCM was removed by rotary evaporation. The polymer was then held under high vacuum for 18 hours to remove trace amounts of monomer and solvent. To confirm structure and determine monomer ratios in the final polymer, NMR analysis was performed for each material.

All coatings were made using PDMS of 26000 g/mol molecular weight with 1 wt% bismuth catalyst, 0.25 wt% poly(diethyl siloxane) (crosslinker), and dissolved in ethyl acetate at 0.2g/mL. The solutions were mixed into a homogeneous solution. Slides were initially spray coated with the PDMS solution with no additive polymers to provide a thin adhesion layer. The coating was allowed to dry for 24 hours, and then was cured at 120 °C for 24 hours under vacuum. The solution containing the amphiphilic block copolymers was pipetted onto the glass slides at this point, and allowed to dry for 48 hours. After this time the slides were cured at 120 °C for 24 hours, yielding a coating roughly 1mm thick at the surface.

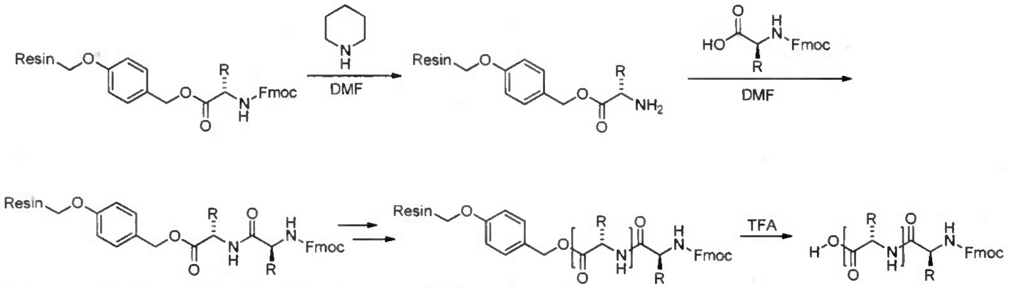
3) Synthesis of PS-*b*-P(DMS-*stat*-MVS) diblock copolymer with grafted oligopeptides. The synthesis of PS-*b*-P(DMS-*stat*-MVS) *via* siloxane trimer ring opening polymerization was performed under anionic conditions (scheme 6). The alkyl and PEG modified lysine was synthesized over three steps (scheme 7). First the alcohols were oxidized using Jones' reagent. Then the carboxylic acids were converted to activated esters using N-hydroxysuccinimide. The activated esters were then reacted with lysine to produce the modified-lysines. The cysteine-modified-lysine oligopeptides were synthesized by solid phase peptide synthesis to produce both the block and alternating oligopeptides. A generalized method of solid phase peptide synthesis is shown in scheme 8. The carboxylic acid of the oligopeptide was then methylated. PS-*b*-P(DMS-*stat*-MVS) was modified with one of the two oligopeptide to produce oligopeptide-functionalized PS-*b*-P(DMS-*stat*-MVS) diblock copolymer by thiol-ene click chemistry (scheme 9).



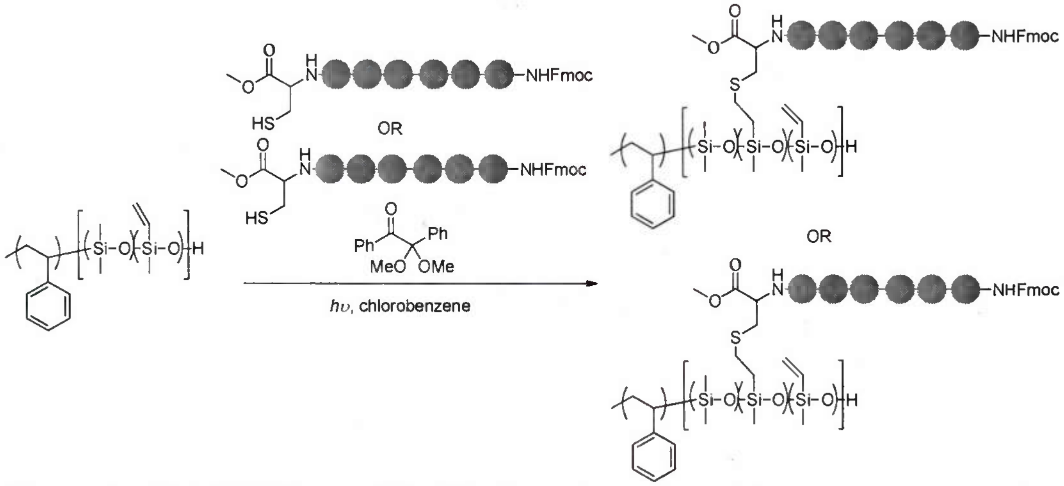
Scheme 6. Hexamethylcyclotrisiloxane ring opening anionic polymerization.



Scheme 7. Synthesis of both cysteine-modified-lysine oligopeptides.

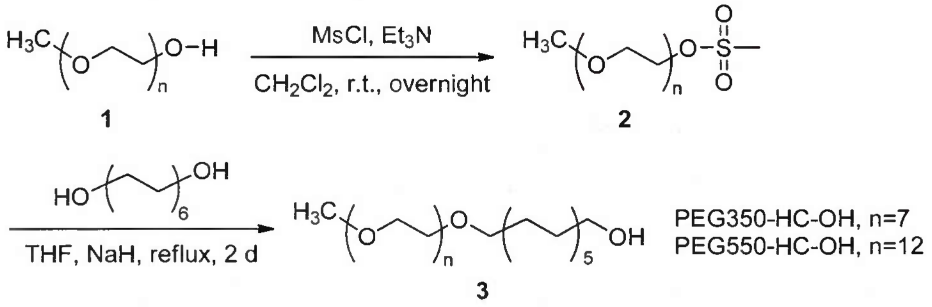


Scheme 8. Generalized solid phase peptide synthesis.

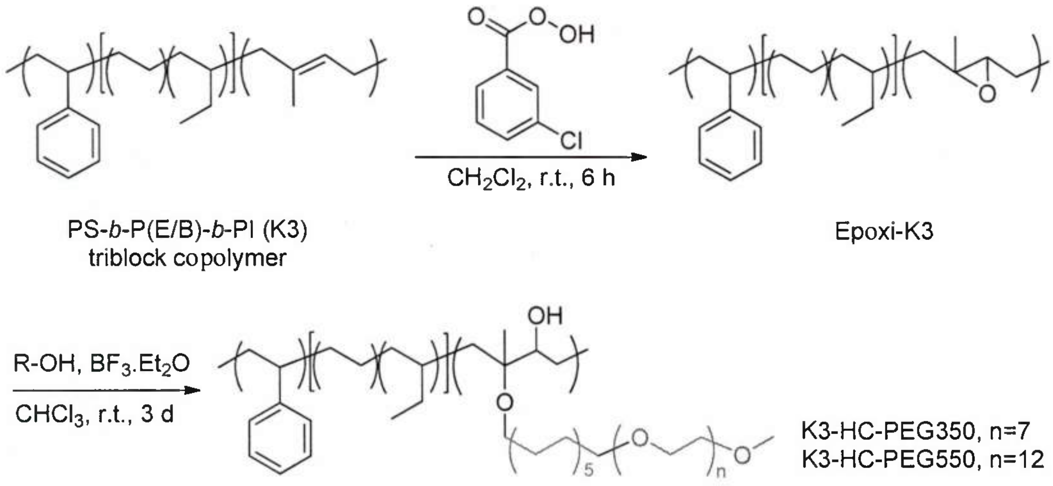


Scheme 9. PS-*b*-P(DMS-*stat*-MVS) diblock copolymer is modified with cysteine-modified-lysine oligopeptide which was performed *via* thiol-ene chemistry.

4) Synthesis of fluorine-free, non-ionic amphiphilic PS-*b*-P(E/B)-*b*-PI triblock copolymers. The fluorine-free, hydrocarbon/PEG based surfactants were synthesized through nucleophilic substitution reaction of hydrocarbon (12 carbons) and PEG derivatives (Scheme 10). The surfactants were then covalently attached to PS-*b*-P(E/B)-*b*-PI polymer through a simple two step functionalization chemistry (Scheme 11). In the first step, poly(isoprene) was oxidized to introduce epoxy-rings on the polymer backbone. This step was followed by catalytic ring-opening and etherification by the hydroxyl groups from the hydrocarbon/PEG surfactants.



Scheme 10. Synthesis of PEGylated hydrocarbon amphiphilic side chains.



Scheme 11. Epoxidation and subsequent catalytic ring-opening etherification of PS-*b*-P(E/B)-*b*-PI to provide final functionalized polymers with amphiphilic structures.

Settlement and removal of *Ulva*. *Leaching:* Test slides were leached in a 30 L tank of recirculating deionized water for at least 24 hours. The slides were equilibrated with seawater for one hour prior to the start of the experiment. ***Settlement of spores:*** Zoospores were obtained from mature *Ulva* plants by the standard method. Zoospores were settled in individual dishes containing 10 ml of zoospores (1.0 x10^6 ml^{-1}) in the dark at ~20°C. After 1 h the slides were gently washed in seawater to remove unsettled zoospores. Slides were fixed using 2.5% glutaraldehyde in seawater. The density of zoospores attached to the surfaces was counted on each of 3 replicate slides using an image analysis system attached to a fluorescence microscope. Spores were visualized by autofluorescence of chlorophyll[10]. Counts were made for 30 fields of view (each 0.17 mm^2) on each slide. ***Strength of attachment of spores:*** Slides settled with zoospores for 1 hour by the above method, were exposed to a shear stress of 53 Pa created by the turbulent flow of seawater in a specially designed water channel. Following this, slides were fixed in gluteraldehyde as described above. The number of spores remaining attached was compared with unexposed control slides (the same as used to determine settlement). The number of cells in 30 fields of view (each 0.17 mm^2) was counted on each of 3 replicate slides. ***Growth of sporelings:*** Spores were settled on 6 replicate slides of each sample at the same time and from the same batch as those in the settlement assay, but were grown for 7 days to the stage of sporelings (young plants). Sporeling biomass was determined *in situ* by measuring the fluorescence of the chlorophyll contained within its cells in a Tecan fluorescence plate reader. The biomass was quantified in terms of relative fluorescence units (RFU). The RFU value for each slide is the mean of 70 point fluorescence readings. The data are expressed as the mean RFU of 6 replicate slides; bars show SEM (standard error of the mean). ***Attachment strength of sporelings:*** The water jet was used to measure strength of attachment of the sporelings[11]. One of each of the six replicate slides was subjected to a single impact pressure by the water jet. A series of water pressures was used and the proportion of biomass removed was estimated using the fluorescence plate reader.

Attachment and removal of *Navicula Diatoms*. *Leaching:* All coatings were leached in deionized water for 48 hours prior to testing. Following this, all coatings were immersed in seawater for one hour before the start of the experiment. ***Initial attachment (density of cells attached after gentle washing):*** *Navicula* cells were cultured in F/2 medium contained in 250 ml conical flasks. After 3 days the cells were in log phase growth. Cells were washed 3 times in fresh medium before harvesting and diluting to give a suspension with a chlorophyll a content of approximately $0.25\ \mu g\ ml^{-1}$. Cells were settled in individual dishes containing 10 ml of suspension at ~20°C on the laboratory benches. After 2 h the slides were exposed to a submerged wash in seawater to remove cells which had not properly attached (the immersion process avoided passing the samples through the air-water interface). Samples were fixed in 2.5% glutaraldehyde, air dried and the density of cells attached to the surface was counted on each slide using an image analysis system attached to a fluorescence microscope. Counts were made for 30 fields of view (each $0.064\ mm^2$) on each slide. ***Strength of attachment:*** Slides settled with *Navicula* were exposed to a shear stress of 52 Pa in a water channel. Samples were fixed and the number of cells remaining attached was counted using the image analysis system described above.

Results and Discussion

1) Patterned Surfaces. Epoxide terminated PAGE (PAGE-Epoxide) was first synthesized by the reaction of PAGE-OH with epichlorohydrin in the presence of NaH. Figure 1 shows the ^{1}HNMR spectrum for PAGE-OH after epoxidation and purification. The signals at $\delta = 3.26$, 2.91, and 2.70 ppm were assigned to the protons of the epoxide end group, in agreement with the expected structure. The signal at 4.56 ppm is characteristic of the methylene protons of the initiator, whereas the peak at 4.56 ppm is typical of the -CH_2- protons of benzyl alcohol. The epoxidation yield could be estimated from the relative integral value of the peaks at 3.26 and 4.56 ppm. This ratio was close to 1:2, consistent with a quasi-quantitative conversion of the hydroxyl end group of PAGE into an epoxy one.

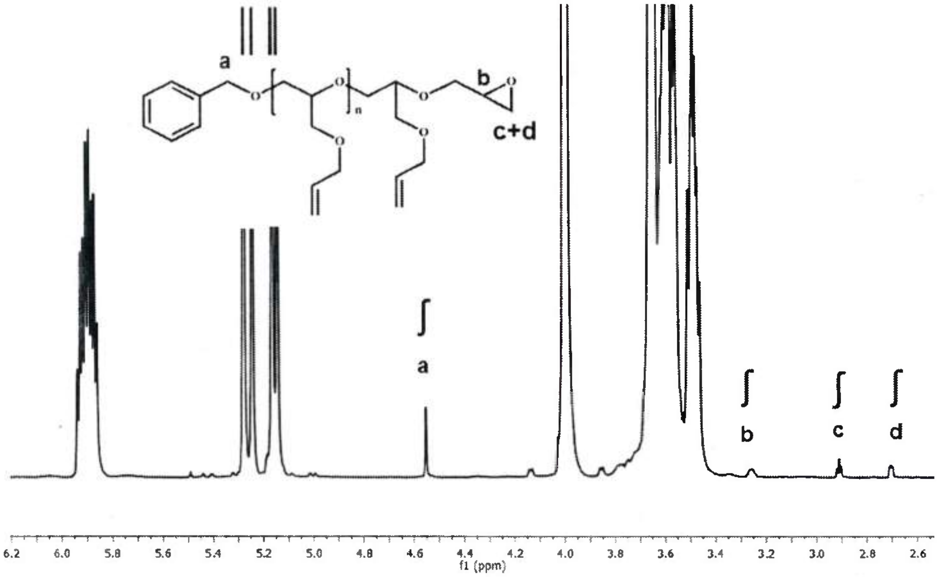
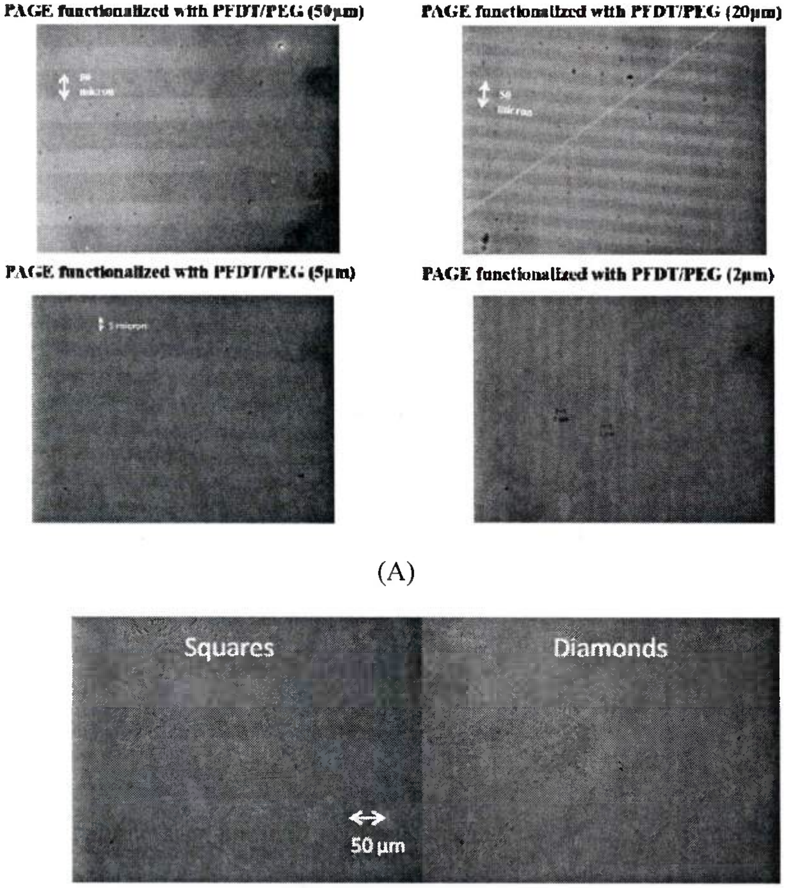


Figure 1.^{1}H NMR spectrum of Epoxide-PAGE in $CDCl_3$.

Hydrophobic/hydrophilic patterned PAGE brushes with stripes of width 50 to 2 microns were produced using UV photolithography. Figure 2 shows optical microscope images of these patterns, the dark stripes are believed to be areas coated with the hydrophobic PFDT side groups.

Figure 2 shows a SIMS image of the 50 micron patterned surfaces, the bright strips are areas containing fluorine, and therefore confirm the patterning of the PAGE brushes with PFDT. 50 micron and 20 micron samples were prepared and sent to the Callows, at the University of Birmingham for Ulva zoo spore biofouling assays. The results for the Ulva zoospore settlement assays can be seen in Figure 4. Figure 4 shows an optical image of the settlement of zoo spores and show that the zoo spore prefer to settle on one chemical moiety over the other. This is evident in their settling in stripes, presumably induced by the underlying chemical pattern. We are also able to achieve not only stripe patterns but also square and diamond patterns, as shown in Figure 2 (B). This will also allow us to test how 2D pattern morphology along with 1D pattern length scale affects the adsorption of various marine organisms such as Ulva zoospores and barnacle cyprids.



(A)

(B)

Figure 2. (A) Microscope images of 50, 20, 5 and 2 micron PFDT/PEG patterned PAGE brush surfaces. (B) Square and diamond patterned surfaces produced by UV double exposure thiol-ene click chemistry.

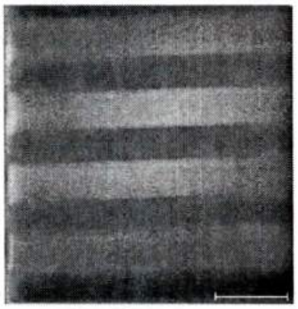
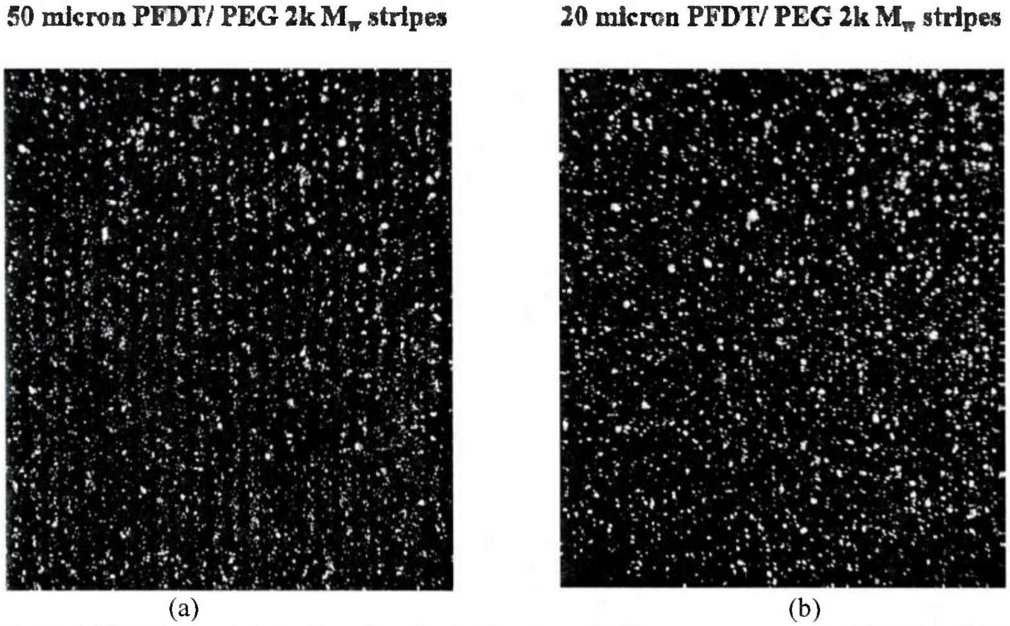


Figure 3. SIMS image of 50 micron PFDT/PEG patterned surface. Light areas indicate the presence of fluorine.



50 micron PFDT/ PEG 2k M_w stripes

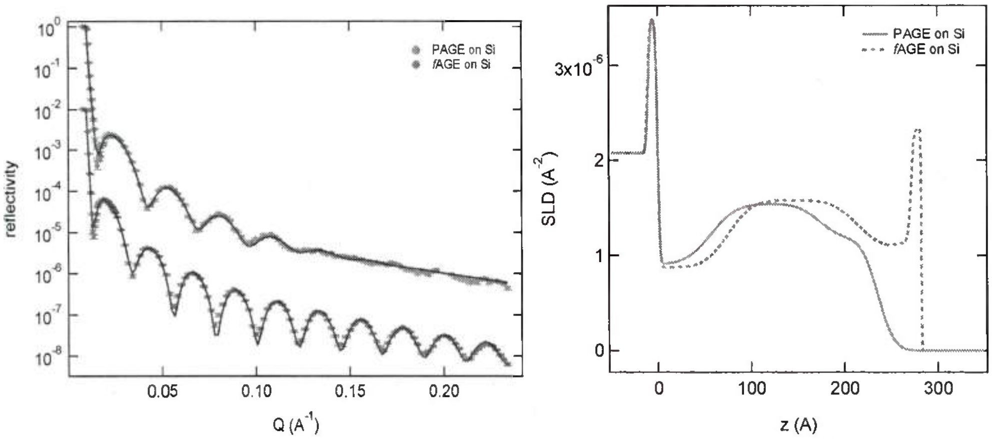
20 micron PFDT/ PEG 2k M_w stripes

(a)

(b)

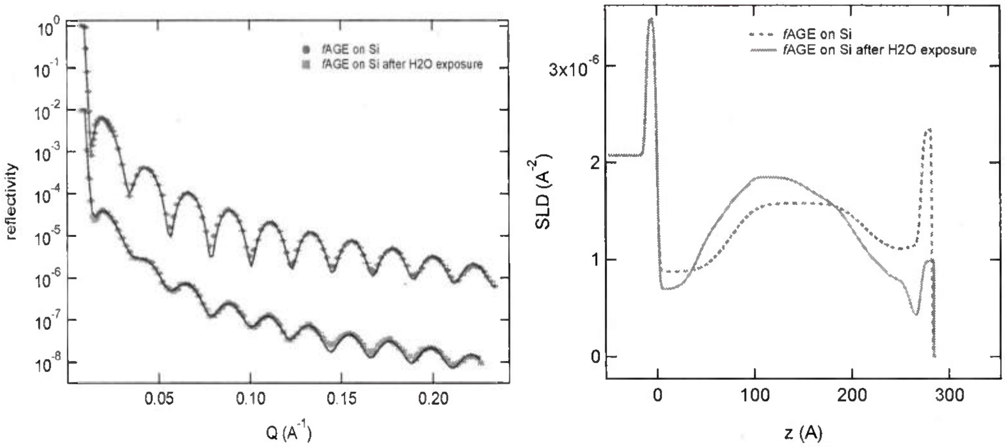
Figure 4.Microscope image showing the settlement of Ulva zoospores onto (a) 50 μ, (b) 20 μ PFDT/PEG 2k M_w stripe patterned surfaces.

Neutron reflectivity (NR) measurements were taken of the PAGE and fAGE polymer brushes before and after immersion in water (H_2O and D_2O). This was done in order to see how the surfaces rearranged themselves after prolonged periods submerged in water, as would be the case on the hull of a ship. This rearrangement is significant to our understanding of how ambiguous surfaces interact with the marine environment. This work also demonstrates the usefulness of NR in determining the structure of antifouling coatings in situ. Figure 5 (a) shows the reflectivity profiles for a PAGE polymer brush before functionalization and after functionalization with PFDT. Figure 5 (b) shows the scattering length density profiles for these two surfaces. The sharp contrast between the SLD of the PFDT (fluorine has about the same high scattering length as ^2H) and PAGE enables the position of the PFDT side groups in these dry layers to be resolved, and reveals as expected due to their low surface energy that these groups are concentrated at the very edge of the brush/air interface. These SLD profiles are being analyzed to determine the initial composition versus depth profile of the brush.



(a) (b)

Figure 5. Neutron reflectivity versus normal component of the neutron wave vector (a) and scattering length density profile (b) for a PAGE polymer brush on a 20 nm APTMS underlayer and fAGE polymer on the same underlayer, taken in air.



(a) (b)

Figure 6. Neutron reflectivity and scattering length density profile for a fAGE brush before and after exposure to D_2O for 4 days, H_2O for one additional day and then allowed to dry for 1 day. Annealing for 12 hours at 90 °C did not change the NR curve for the dried sample.

Figure 6(a) shows the change in the neutron reflectivity curves of a PAGE brush after functionalization with PFDT (fAGE brush) before and after immersion in water, Figure 6 (b) shows the predicted SLD profiles for the measured neutron reflectivity curves. It is clear that the amount of PFDT at the surface is dramatically reduced whilst the SLD of the aminosilanes has increased, after exposure to water. This suggests that the PFDT side groups are burying themselves within the coating interface as well as the crosslinked APTMS layer and not returning to the surface upon drying.

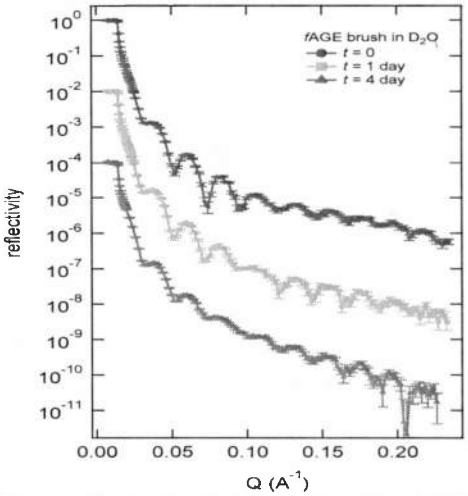
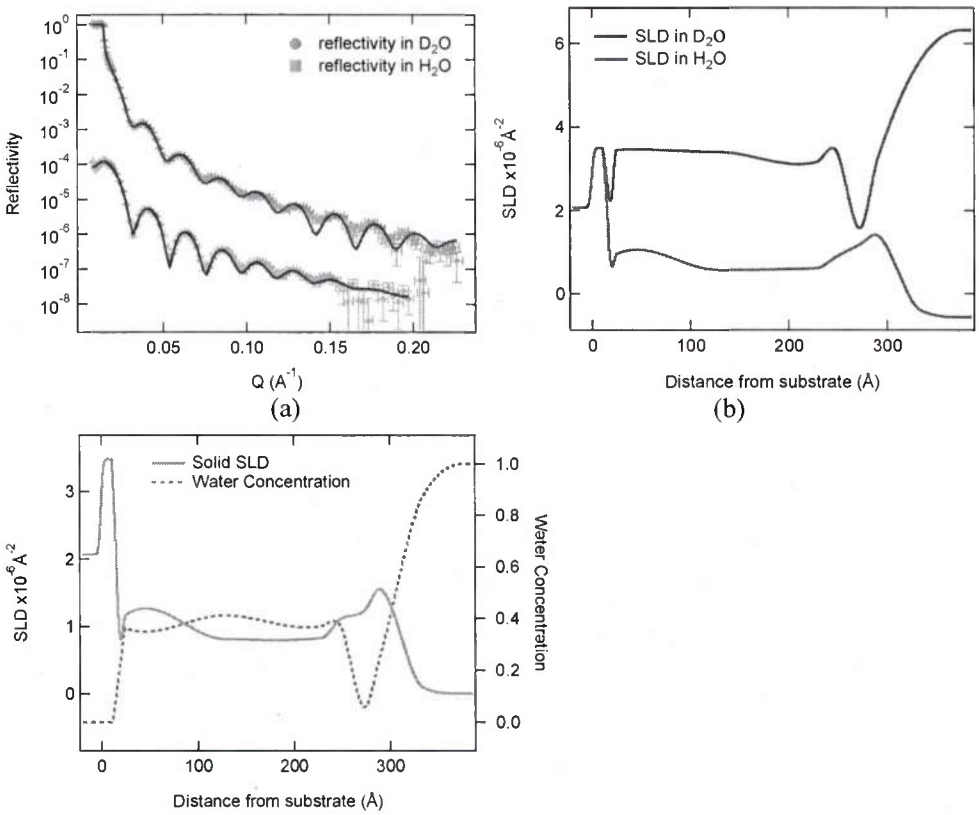


Figure 7. Reflectivity profile for fAGE brush in D_2O after 0, 1 and 4 days.

The same fAGE brush was immersed in D_2O in order to investigate the penetration of water into the brush. Figure 7 shows how the neutron reflectivity curves change over 4 days of exposure. The change in the reflectivity curves indicates the reorganization of the brush and its cross linked APTMS layer caused by absorption of D_2O into the film. This time dependent reflectivity change demonstrates the relatively long time scales over which the surface coating reorganizes; fitting to obtain the scattering length density profiles at the intermediate times is ongoing. Some antifouling coatings may require prolonged presoaking in water before exposure to foulants in order to reliably determine their long term antifouling efficacy.



(a)

(b)

(c)

Figure 8. (a) Reflectivity curves for fAGE brush immersed in H_2O and D_2O. (b) predicted SLD profile for fAGE brushes immersed in D_2O (4 days) and subsequently in H_2O for one extra day.

(c) Solid SLD profile of fAGE brush plotted on top of the approximate water concentration profile.

The NR curves for the fAGE brush in D_2O (after 4 days) and then after exchanging H_2O for D_2O, shown in Figure 8 (a), were compared in order to elucidate any structure information. The water concentration, as seen in figure 8 (c) could be approximately determined from the SLD profiles shown in figure 8 (b) assuming that the concentration profiles of D_2O and H_2O are identical . It is clear that the APTMS layers clearly contain large amounts of water (on the order of 40%) when immersed.. Interestingly the water content appears to drop nearly to zero where the reorganized fAGE brush units reside. We expect to use NR measurements in the future to determine how amphiphilic P(EO-co-fAGE) polymer brushes rearrange when put in water.

2) PDMS-Based Amphiphilic Block Copolymer Coatings

A systematically prepared set of block copolymers produced for this study was first analyzed by NMR and GPC to determine the percentage of PEG and fluorinated content as a percentage of block copolymer content as well as to determine the overall molecular weight. A representative ^{1}HNMR of one of the block copolymers is shown in Figure 9. The peak at δ0 relates to the PDMS component of the block copolymer. The peaks at δ4.15 and δ4.35 come from both AF6 and PEGMA and are used to determine the ratio of PDMS:PEGMA+AF6. Then the relative ratio of PEGMA:AF6 is determined using those peaks and comparing to the peak at δ3.6, which comes from the PEG component of the PEGMA. From these relative integrations, a complete composition of the block copolymers was determined.

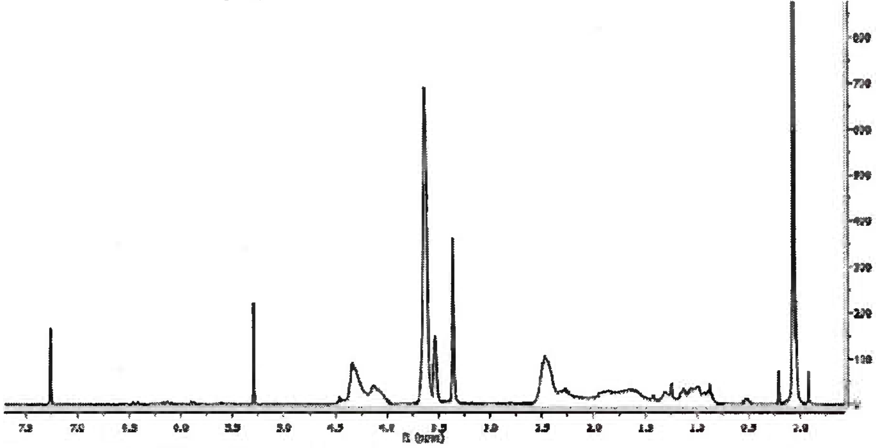


Figure 9: 1H NMR of PDMS based block copolymer

Each polymer produced was loaded into the PDMS matrix at both 1wt% and 4wt%, to produce two coatings per block copolymer. A list of samples, including the composition of the block copolymers used as well as the sample loading is included in Table 2. Listed is the monomer:initiator ratio used for each polymerization, and the content of the three components of the block copolymer as well as the block copolymer loading into the PDMS matrix for each coating.

Sample	Mon-Init	PDMS%	PEGMA %	AF6%	Copoly %
UP25	30-1	78	14	8	1
UP26					4
UP27		79	11	10	1
UP28					4
UP29		86	6	8	1
UP30					4
UP41	60-1	70.0	21.3	8.7	1
UP42					4
UP43		72.3	13.7	14.0	1
UP44					4
UP45		74.4	9.9	15.7	1
UP46					4
UP47	150-1	53.0	35.6	11.4	1
UP48					4
UP49		52.8	24.7	22.5	1
UP50					4

Table 2: List of all coating samples and block copolymer content

Figure 10 shows a representative GPC measurement used to determine the molecular weight of each of the samples. All GPCs were run in tetrahydrofuran (THF). From the GPC, it was determined that a monomer:initiator ratio of 30:1 produced a polymer of molecular weight 17 kg/mol, a ratio of 60:1 gave 21 kg/mol, and a ratio of 150:1 gave 30 kg/mol.

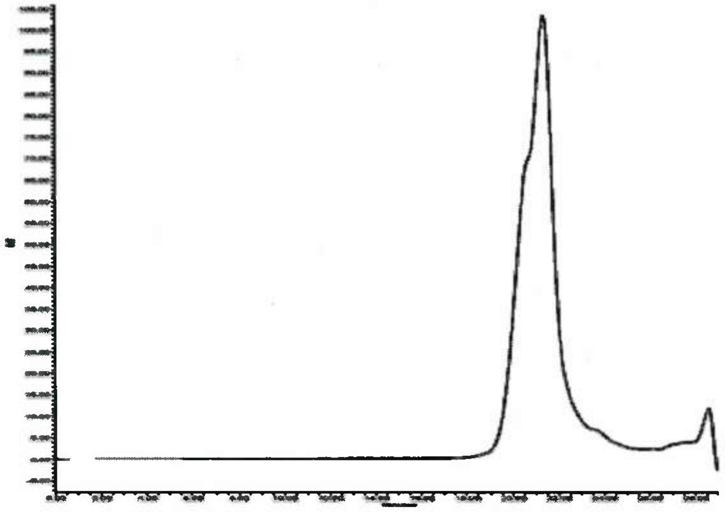


Figure 10: GPC trace of PDMS based block copolymer

For each of these coatings, settlement and removal testing of *Ulva* was done by the University of Birmingham to study the antifouling and fouling release performance of these materials. As a reference, a PDMS sample prepared in the same way as the other coatings, but without a block copolymer additive, was used. The tests were done at two different days, one test including UP25-UP30, and the other including UP41-UP50 due to limits of testing many samples during the same week. Initial settlement of *Ulva* was performed, then incubated for one week while feeding every other day. During this incubation period, several of the samples, including UP28 and UP30 exhibited "spontaneous removal" or sporelings. Under conditions of incubation and feeding of the sporelings, they began to detach in significant quantities and float off the coatings. Figure 11 shows the sporeling attachment data as taken by TECAN measurement after incubation. Overall, most coatings showed higher initial settlement than standards used, with the exception of those exhibiting spontaneous removal of the sporelings

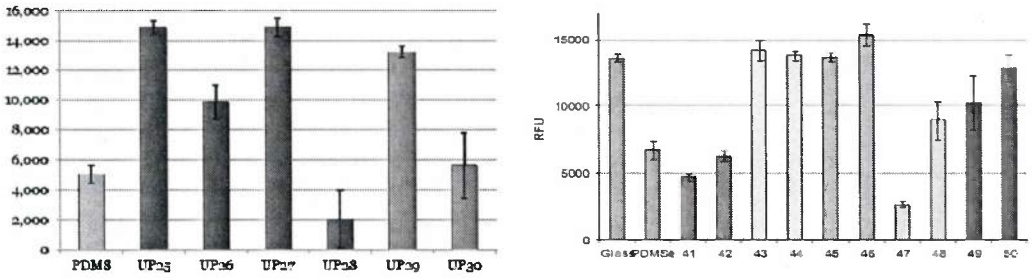


Figure 11: *Ulva* sporeling attachment data

After incubation, the coatings were subjected to removal testing in a delaminated flow chamber at the University of Birmingham. All samples were subjected to 8Pa of shear stress, which is the lowest flow rate attainable on the setup used. After 5 minutes of removal at the flow rate, the samples were removed and again analyzed using TECAN to determine sporeling density on the coatings, shown in Figure 12. The best performers of the first set were UP28 and UP30, showing near 100% removal after the test. These were also the samples exhibiting the spontaneous removal during incubation. In the second group, UP45 and UP46 were the best performers, again showing near 100% removal of sporelings.

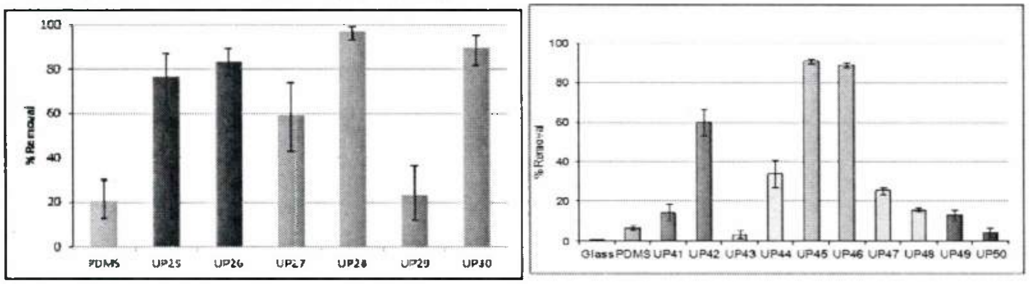


Figure 12: Percentage Removal of *Ulva* sporelings

For the tests performed, the low molecular weight polymers were overall better performers than the high molecular weight materials, but required a higher loading of the block copolymer. This is likely due to the lower PEG and fluorinated content of the lower molecular weight materials. Additionally, it was noted that for low molecular weight materials, a 50:50 and 30:70 ratio of PEGMA:AF6 showed the best performance, while at mid range molecular weights only the 30:70 ratio showed high performance, indicating the importance of the chemical composition of the amphiphilic block copolymers.

Next, these coatings were taken for analysis by NEXAFS at Brookhaven National Labs. This technique allows for the investigation of the chemical bonding environments at the surface of the coatings. From this, the relative composition of coatings can be compared as well as any orientation of chemical groups as the surface. Figure 13, Figure 14, and Figure 15 all show NEXAFS plots. Figure 13 shows a scan of several angles for UP30 to determine surface orientation of chemical groups on the coating. The fluorinated component of these surfaces can be seen to be oriented by the C-F bond shown on the spectrum. Because the intensity of the peak increases with increasing angle, the C-F bonds are oriented parallel to the surface meaning that the C-C bonds associated with the fluorinated chains are oriented perpendicular to the surface. For all spectra, three peaks are labeled, a C-Si peak associated with the PDMS matrix, a C-O peak associated with the PEG content, and a C-F peak associated with the fluoroalkyl content.

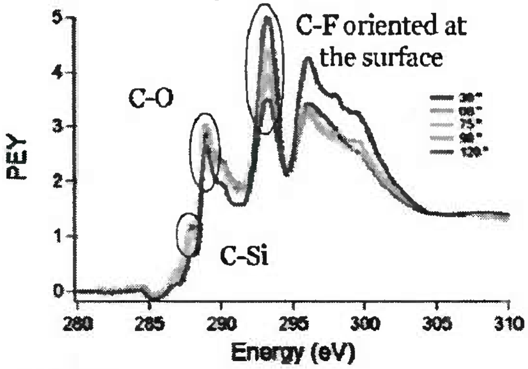


Figure 13: NEXAFS plot of UP30

The next plot shows a comparison of UP42, UP44, and UP46, all samples with molecular weight of 21kg/mol, and a sample loading of 4% in the PDMS matrix. However, the samples differ by the content of the PEG and fluorinated components. As can be seen, with increasing fluorine content there is an increase in the intensity of the C-F bond and a decrease in the C-O bond associated with the PEG groups. The legend lists the ratio of AF6:PEGMA for the three copolymer containing samples.

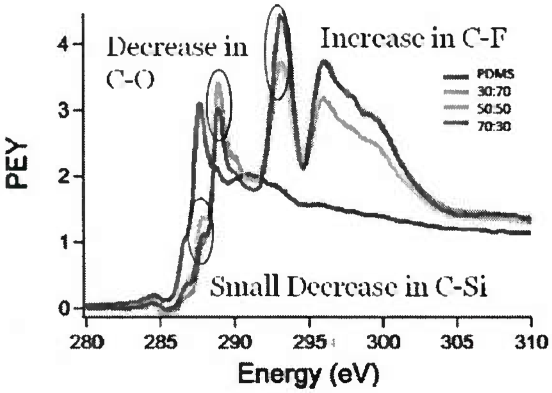


Figure 14: NEXAFS Spectra of PDMS, UP42, UP44, and UP46

The final spectrum shows the surface rearrangement of UP28 after immersion in water. The two spectra show the standard sample as well as a coating which had been immersed in deionized water for 5 days prior to NEXAFS analysis. The sample was then quickly removed from water and analyzed. As can be seen there is significant rearrangement of the surface after immersion. There is an increase in both the PEG and fluorinated content in the immersed sample as well as a decrease in the PDMS, indicating that the block copolymers strongly surface segregate in aqueous environments.

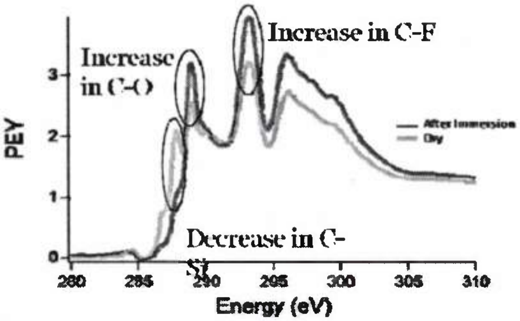


Figure 15: NEXAFS Spectra of UP28 before and after immersion in water

This work demonstrated a systematic study of the composition of amphiphilic block copolymers as additives for PDMS based coatings to determine which candidates were most effective and to analyze surface properties of these coatings. It was observed that in general lower molecular weight block copolymers exhibited better fouling release properties, and that for these systems, the fluorine content needed to be as high or higher than the PEG content to produce excellent fouling release. These coatings were shown to be highly effective, demonstrating very low attachment strength of the *Ulva* sporelings, requiring very minimal energy to remove them and clear the surfaces.

3) Unnatural oligopeptide surfactants PS-*b*-P(DMS-*stat*-MVS) diblock copolymers.

The goal of this work is to further the study of oligopeptide surfactant PS-*b*-P(DMS-*stat*-MVS) diblock copolymer for marine antifouling/fouling release applications. PS-*b*-P(DMS-*stat*-MVS) diblock copolymer was obtained as shown above in scheme 6. The PEG modified lysine amino acid was synthesized with a yield of 34% over three steps (scheme 7). The alkyl modified lysine amino acid was synthesized with a yield of 65% over three steps (scheme 7). In both cases, the modified lysine was purified using column chromatography after the final step. Solid phase peptide synthesis and methylation was performed to produce the block and alternating cysteine-hexyl-modified-lysine oligopeptides (scheme 8). The oligopeptide after being purified with a titration in hexane was obtained with a yield of 71% and 75% for the block and alternating oligopeptides respectively. The solid phase peptide synthesis method performed in a multi-gram synthesis for each single batch. Both of the oligopeptides were attached to the diblock copolymer via thiol-ene "click" chemistry. The modified diblock copolymer was purified by being dissolved in dichloromethane and precipitation in methanol producing a yield of 73% and 69% for the block and alternating oligopeptide respectively (scheme 9).

^{1}HNMR analysis was used to confirm the structure of the unnatural oligopeptide surfactants diblock copolymer. Figure 16 shows the ^{1}HNMR data of the block and alternating modified diblock copolymers from scheme 7 respectively. The new peaks in the block and alternating samples between 3-4.5 ppm are from the alpha carbons in the oligopeptides, directly adjacent to the carbonyls, and the PEG chains from the modified oligopeptides. The peaks around 8 ppm is from the Fmoc protecting group on the oligopeptide chain. The small number of peaks around 6 ppm suggests the lack of alkene functionality of the MVS block in the diblock copolymers. This indicates that most of the alkenes from the diblock copolymer have reacted via thiol-ene click chemistry to attach the oligopeptide chains.

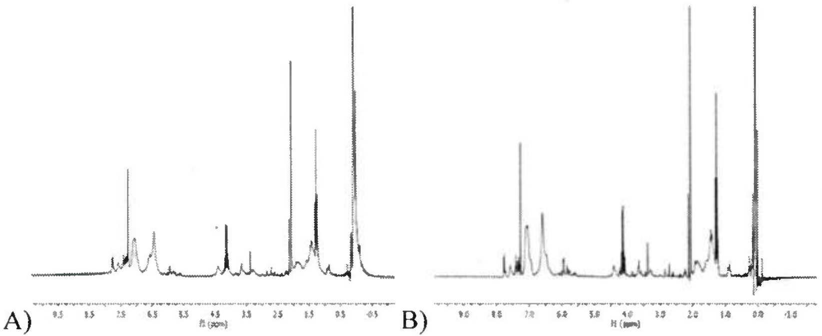


Figure 16. A) ^{1}HNMR spectra of the block oligopeptide attached to the PDMS based block copolymer. B) ^{1}HNMR spectra of the alternating oligopeptide attached to the PDMS based block copolymer

Underwater bubble contact angles were used to monitor the dynamic surface capability of facile reordering of the oligopeptide side chains. The angles dropped to a small degree indicating that the surface was still hydrophobic after rearranging in water (Figure 17A). After 3 days of immersion in DI water, the contact angles reached equilibrium values, suggesting that the surface reconstruction occurred over a period of days. The decrease of the contact angle may be attributed to the molecular reorganization of the surface, which can occur by the migration of the oligopeptide side chains towards the interface of the water-polymer interface. The underwater bubble contact angles dropped to a small degree indicating that the surface was still hydrophobic after rearranging. The polymer coated surfaces were also exposed to fluorescence labeled protein solution (FITC-BSA) to test the polymers against non-specific protein absorption (Figure 2B). The protein absorption for the block and alternating samples was lower than the unmodified control and the SEBS internal standard.

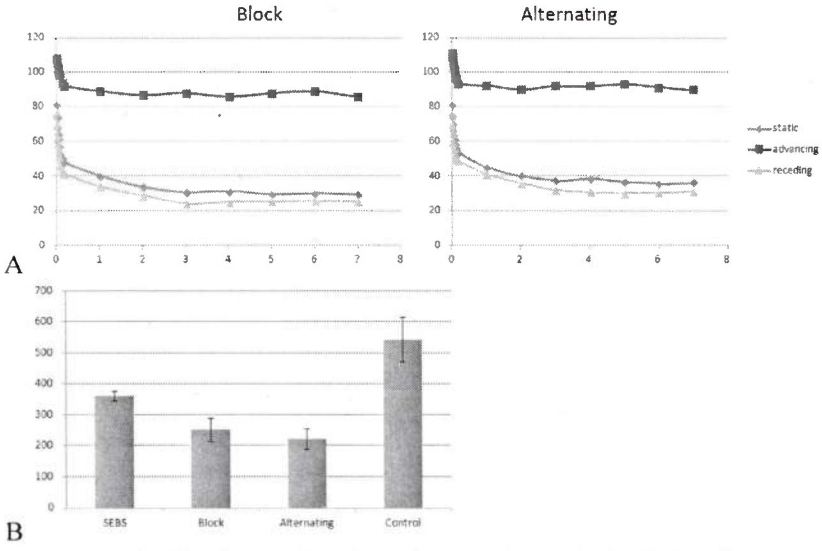


Figure 17. A) Captive air bubble contact angles and B) relative fluorescence intensities of BSA-FITC adsorbed on oligopeptide side chain modified PS-*b*-P(DMS-*stat*-MVS) in comparison to SEBS base layers.

All *Ulva* assays were performed in the laboratory of Dr. Maureen Callow at the University of Birmingham, UK. Spore settlement density on the alternating and the block oligopeptide diblock copolymer coatings was about half of the PDMSe standard and significantly lower than the unmodified control. Sporeling growth appeared normal on all coatings indicating that toxicity was not an issue, with the block twice lower than the alternating sample (Figure 18A). The block and alternating samples had a similar percent removal of sporelings due to a shear stress of 52 Pa (Figure 18B). Their percentage removal was several times higher than the unmodified control, indicating that the oligopeptides significantly increased the fouling release of the diblock copolymer (Figure 18C).

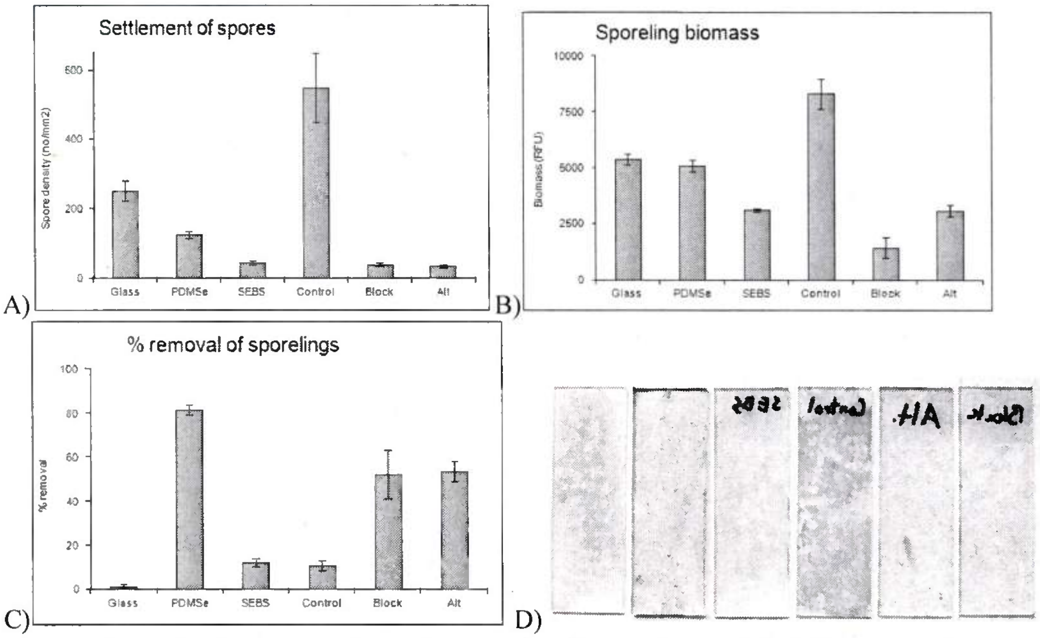


Figure 18. A) The Settlement of sporelings on the oligopeptide-modified diblock copolymer after 7 days. B) The biomass of sporelings on the oligopeptide modified diblock copolymer coatings after 7 days. C) Percent removal of 7 day old sporelings from the oligopeptide modified diblock copolymer coatings due to a shear stress of 52 Pa.

4) Hydrocarbon/PEG amphiphilic surfactants modified PS-*b*-P(E/B)-*b*-PI triblock copolymers.

The goal of this current work has been to study new hydrocarbon/PEG surfactants modified PS-*b*-P(E/B)-*b*-PI triblock copolymer for marine antifouling/fouling release applications. Synthesis of the target polymers was carried out into two parts, the ether linkages of each coupling step was chosen to induce resistance towards various forms of metabolism, and the details of each step were depicted above in schemes 10 and 11. In the first part (scheme 10), the hydrocarbon-block-poly(ethylene glycol), abbreviated as PEG-HC-OH, is composed by a mono-disperse hydrocarbon block attached to a PEG block through an ether linkage. The reaction takes place with high conversion, and mono-substituted product was isolated using column chromatography in good yield, with 89%, 95% yield for PEG350-HC-OH and PEG550-HC-OH, respectively. Only one of the two hydroxyl groups on the starting 1,12-dodecanediol was reacted with the methylsulfonyl group on PEG chain, resulting in the free-hydroxyl terminated products. The free-hydroxyl groups were to be used for the epoxy-ring opening reaction in the next step, to further couple the products with SEBI triblock copolymer. In the second part of polymer synthesis, double bonds in SEBI triblock copolymer were first converted to epoxy-rings through oxidation reaction of the polyisoprene block in the polymer. This reaction introduced more reactive functional groups which can be further coupled with PEG-HC-OH side chains by ring-opening reaction under the catalysis of BF_3•Et_2O. After three days reaction at room temperature, the resulting polymers were purified by precipitation methods. K3-HC-PEG350 can be precipitated in methanol, but K3-HC-PEG550 showed good solubility in methanol and therefore was precipitated and washed in DI water.

^{1}HNMR, IR, GPC and elemental analysis were used to confirm the reaction product of each step. For example, Figure 19 shows the ^{1}HNMR and IR spectra of the final amphiphilic side chain modified PS-*b*-P(E/B)-*b*-PI (or K3) triblock copolymer compared to the starting materials. The new peaks at 3.3-3.6 ppm in ^{1}HNMR (Figure 19A), and the appearance of stronger peaks at 1000-1200 cm^{-1} (C-O-C stretching), 2929, 2855 cm^{-1} (C-H stretching) in IR (Figure 19B) suggests the successful induction of the hydrocarbon/PEG amphiphilic side chains in the polymer system.

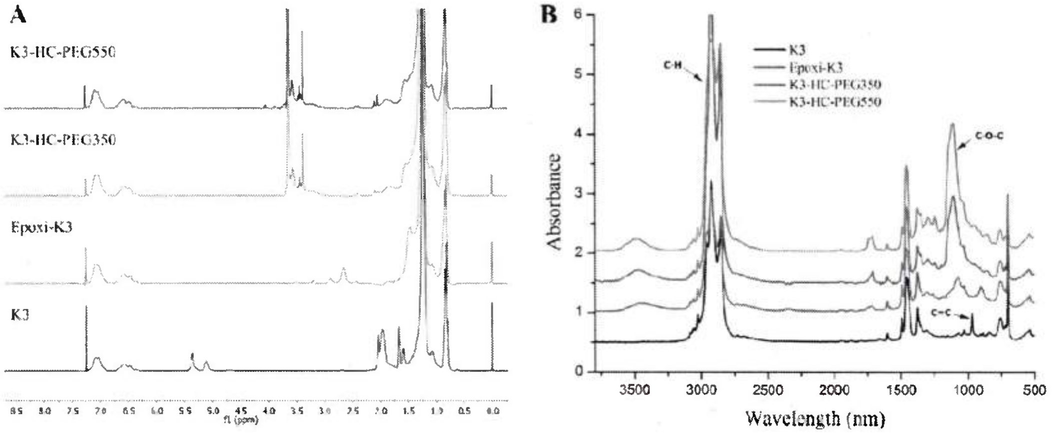


Figure 19. ^{1}HNMR and IR spectra of starting polymer PS-*b*-P(E/B)-*b*-PI (or K3), epoxidized K3 (Epoxi-K3), and amphiphilic side chain modified K3 triblock copolymers (K3-HC-PEG350 and K3-HC-PEG550).

Atomic force microscopy (AFM) was used to characterize the morphology of the surfaces. Figure 20 shows the AFM phase images of the triblock copolymers with grafted amphiphilic side chains that form phase-separated surface patterns. The surface topography is significantly different from previously "Brij ™" modified K3 polymers that showed mostly lying-down cylinder structures , probably because the hydrophilic PEG segment in "Brij ™" modified polymers was linked between two relatively hydrophobic units (long hydrocarbon chains and K3 polymer backbones). In the current structures, however, PEG is further away from those hydrophobic components and therefore can form spherical structures. Since surface morphology is an important factor that can affect many cellular behaviors, AFM images revealed some interesting information related to the different antifouling properties on the hydrocarbon/PEG based polymer surfaces.

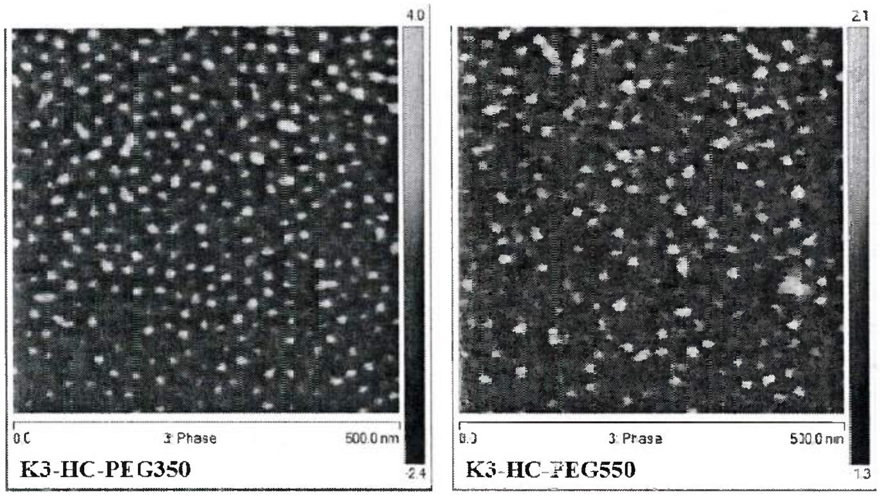


Figure 20. AFM phase images of K3-HC-PEG350 and K3-HC-PEG550 after dry annealing at 120°C for 12 h

Underwater bubble contact angles were also used to monitor the dynamic surface capability of facile reordering of the side chains in those amphiphilic polymer systems. Compared to those on SEBS base layer coated glass slides, the contact angles of the air bubbles on the amphiphilic side chain modified surfaces are significantly lower (Figure 21 A). After 3 days of immersion in DI water, the contact angles reached equilibrium values, suggesting that the surface reconstruction occurred over a period of days. The decrease of the contact angle may be attributed to the molecular reorganization of the surface, which can occur by the migration of the polystyrene block and the hydrocarbon block away from the interface and/or the reorientation of the PEG segments to the water-polymer interface. The polymer coated surfaces were also exposed to fluorescence labeled protein solution (FITC-BSA) to test the polymers against non-specific protein absorption. The results showed that compared to the SEBS base-layer coated surfaces, both hydrocarbon/PEG functionalized triblock copolymers surfaces are more effective against FITC-BSA absorption, while K3-HC-PEG550 shows the lowest absorption. This supports previous findings that the length of PEG unit is a major factor for its "non-adhesive" properties, and longer PEG chain length shows higher resistance against non-specific bindings towards proteins.

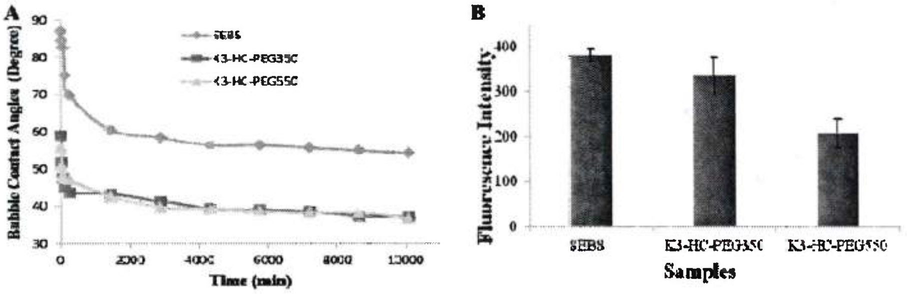


Figure 21. A) Captive air bubble contact angles and B) relative fluorescence intensities of BSA-FITC adsorbed on PEGylated amphiphilic side chain modified PS-*b*-P(E/B)-*b*-PI in comparison to SEBS base layers.

All *Ulva* and *Navicula* assays were performed in the laboratory of Dr. Maureen Callow at the University of Birmingham, UK. Spore settlement density on the hydrocarbon/PEG amphiphilic copolymer coatings was lowest on the K3-HC-PEG550 among all the samples tested (Figure 22A). Observations also suggested that sporelings were so weakly attached to the PEG550 coating that they were detached by movement of the dishes and the water currents this produced. Sporeling growth appeared normal on all coatings and toxicity was not an issue. The percent removal of sporelings due to a shear stress of 52 Pa was approximately 100% from the K3-HC-PEG550 coating (Figure 22B). The ready detachment of sporelings from the PEG550 coating suggests that it would out-perform PDMS at lower shear stresses.

The initial attachment densities of diatoms on the two amphiphilic triblock copolymer coatings were lower than on the SEBS base and PDMSe standards (Figure 22C). Attachment densities were lower on the K3-HC-PEG550 than the K3-HC-PEG350. Diatom removal due to a shear stress of 33 Pa was relatively low from all coatings, however, removal from the K3-HC-PEG550 was higher than from the K3-HC-PEG350 (Figure 22D). The initial attachment of diatoms to both these coatings was relatively low, so the further 20% removal from the K3-HC-PEG550 coating indicates good fouling-release potential.

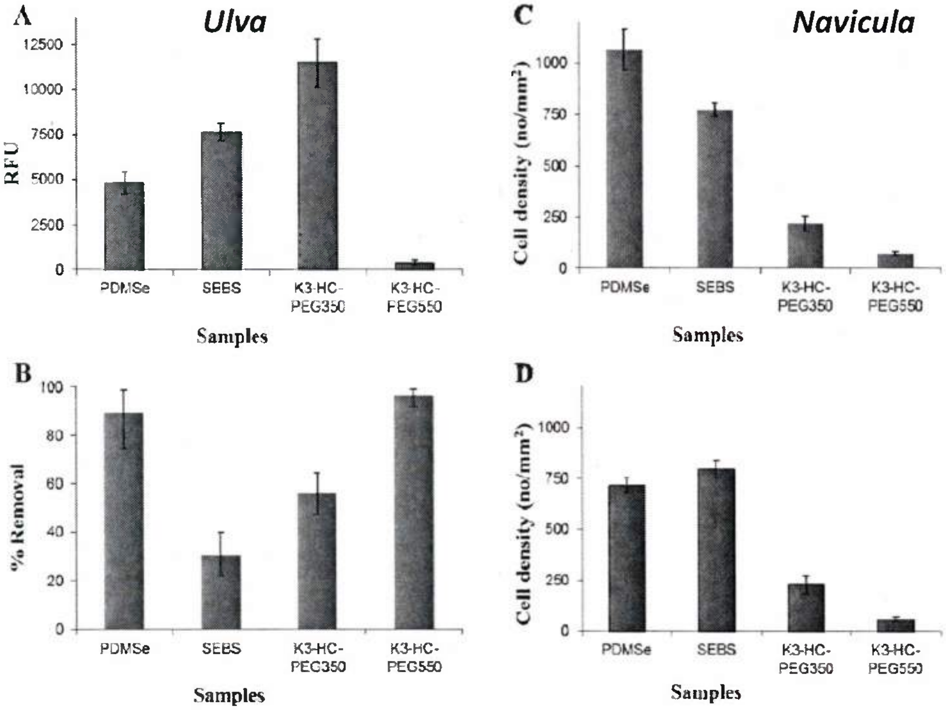


Figure 22. A) The biomass of sporelings on the hydrocarbon/PEG amphiphilic triblock copolymer coatings after 7 days. B) Percent removal of 7 day old sporelings from amphiphilic triblock copolymer coatings due to a shear stress of 52 Pa. C) The density of attached *N. incerta* cells on amphiphilic triblock copolymer coatings after washing. B) The remaining cell densities of *N. incerta* cells from the polymer coatings due to exposure to a shear stress of 33 Pa.

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